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Human and Swine Hosts Share Vancomycin-Resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 Clonal Clusters Harboring Tn*1546* on Indistinguishable Plasmids▽†

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VRE isolates from pigs ($n = 29$) and healthy persons ($n = 12$) recovered during wide surveillance studies performed in Portugal, Denmark, Spain, Switzerland, and the United States (1995 to 2008) were compared with outbreak/prevalent VRE clinical strains ($n = 190$; 23 countries; 1986 to 2009). Thirty clonally related *Enterococcus faecium* clonal complex 5 (CC5) isolates (17 sequence type 6 [ST6], 6 ST5, 5 ST185, 1 ST147, and 1 ST493) were obtained from feces of swine and healthy humans. This collection included isolates widespread among pigs of European Union (EU) countries since the mid-1990s. Each ST comprised isolates showing similar pulsed-field gel electrophoresis (PFGE) patterns (≤6 bands difference; >82% similarity). Some CC5 PFGE subtype strains from swine were indistinguishable from hospital vancomycin-resistant enterococci (VRE) causing infections. A truncated variant of Tn*1546* (encoding resistance to vancomycin) and *tcrB* (coding for resistance to copper) were consistently located on 150- to 190-kb plasmids (rep_{pLG1}). *E. faecium* CC17 (ST132) isolates from pig manure and two clinical samples showed identical PFGE profiles and contained a 60-kb mosaic plasmid (rep_{Inc18} plus rep_{pRUM}) carrying diverse Tn*1546*-IS*1216* variants. The only *Enterococcus faecalis* isolate obtained from pigs (CC2-ST6) corresponded to a multidrug-resistant clone widely disseminated in hospitals in Italy, Portugal, and Spain, and both animal and human isolates harbored an indistinguishable 100-kb mosaic plasmid (rep_{pRE25} plus rep_{pCF10}) containing the whole Tn*1546* backbone. The results indicate a current intra- and international spread of *E. faecium* and *E. faecalis* clones and their plasmids among swine and humans.

Vancomycin-resistant enterococci (VRE) are among the most common nosocomial pathogens in the United States and in several European Union (EU) countries (23, 50). They have frequently been isolated from farm animals, pets, and retail food products in Europe, but until very recently, the detection of VRE from either processing or production food animal environments in the United States was infrequent (11, 20, 42, 53). There is limited evidence as to the direct role of the food chain in the dissemination of VRE among humans. Despite this, the potential hazard has been widely recognized and led to the adoption of intervention measures, such as the ban on the growth-promoting use of antimicrobials in the EU. A remarkable reduction in the prevalence of VRE among animals and humans has been observed after the EU withdrawal (see reference 42 and references therein). However, the role of nonhuman hosts as reservoirs of highly transmissible clones, the transient or permanent human fecal carriage of VRE of animal origin, and the consequent risk of gene transfer to resident human flora are issues still discussed and not fully addressed (41, 42).

Both *Enterococcus faecium* and *Enterococcus faecalis* are opportunistic pathogens comprising some host-specific lineages (30, 53). Strains from human-adapted clonal complexes (CCs) causing most enterococcal infections may eventually be recovered from farm and companion animals (e.g., *E. faecium* clonal complex 17 [CC17] and *E. faecalis* CC2), and strains from CCs commonly found among animals have also been isolated from humans (*E. faecium* CC5, *E. faecalis* sequence type 16 [ST16], or *E. faecalis* CC21) (4, 9, 13, 14, 28, 53). Documented cases of animal-human VRE transmission frequently involve healthy humans in close interaction (farming or petting) with animals, but most of these studies do not provide molecular characterization of either clones or their

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TABLE 1. Origins of VRE isolated from swine and healthy humans during national surveillance studies in Denmark, Portugal, Spain, Switzerland, and the United States (1995 to 2008)

Species	Country	No. isolated from:		Origin	Reference(s)
		Swine	Healthy humans		
E. faecium (n = 40)	Denmark	15	3	15 VREfm from healthy pigs among 1,594 *E. faecium* fecal isolates (DANMAP[a], 1995–2006)	17, 26; this study
				3 VREfm recovered from 525 community-dwelling human samples (2002–2006)	17; this study
	Portugal	4	9	4 VREfm from 84 fecal or environmental samples in different production piggeries (1997; 2006–2007); one isolate from 1997 belongs to the CC5 widespread clone recovered in different countries over years	14, 35
				9 VREfm fecal isolates from 99 healthy volunteers living in different Portuguese cities (2001–2004)	37
	United States	6	0	6 VREfm recently isolated from 55 swine (10.9%) in three Michigan counties (2008)	11
	Spain	2	0	2 VREfm recovered from 900 pig fecal samples at slaughterhouses (9.7% of all pigs slaughtered in 1998) in Valencia and Murcia (1998–2000)	22, 35
	Switzerland	1	0	1 VREfm recovered from samples of pig feces among 155 *Enterococcus* isolates obtained in 16 Swiss farms (1999–2000)	5, 35
E. faecalis (n = 1)	Portugal	1	0	1 VREfs from 84 fecal or environmental samples in different production piggeries (2006–2007)	13

[a] DANMAP, Danish Integrated Antimicrobial Resistance Monitoring and Research Programme.

subcellular genetic elements (1, 3, 10, 17, 26, 28, 31, 33), despite the comprehensive epidemiological studies of Tn*1546* (*vanA*) and Tn*5382* (*vanB*) (8, 24, 38, 52, 54).

In this work, a comparative multilayered molecular analysis of representative VRE strains from swine, healthy humans, and clinical isolates recovered from wide surveillance studies was carried out with the aim of identifying and characterizing epidemic VRE clones and plasmids shared by human and swine hosts.

MATERIALS AND METHODS

Bacterial strains and epidemiological background. The epidemiological background of the 41 isolates analyzed in this study is shown in Table 1. It includes representative isolates of VRE recovered from swine and healthy humans in national surveillance studies conducted in Portugal and Denmark (1995 to 2008) (references 17, 26, 35, 37, and 38 and this study), strains widespread among swine from Switzerland and Spain (5, 22), and the first VRE isolates recently recovered from animals in the United States (11). For comparison, we included a large and well-typed collection of clinical VRE isolates (140 *E. faecium* and 50 *E. faecalis* isolates) recovered from 23 countries, including Portugal, Spain, Denmark, and the United States, during the last 3 decades, most of which had caused hospital outbreaks (12, 16). Testing of susceptibility to 12 antibiotics was performed either by E strip (bioMérieux, Solna, Sweden) or by a standard agar dilution method following recommended guidelines of the manufacturer or CLSI (6). The presence of genes coding for antimicrobial (glycopeptides, macrolides, tetracyclines, and aminoglycosides) and copper (*tcrB*) resistance and putative virulence traits (*agg*, *gel*, *cyl*, *esp*, and hyl_{Efm}) was analyzed by using different PCR schemes (18, 36).

Clonal relatedness. Clonal relatedness was established by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), as described previously (36, 48; http://www.mlst.net). Computer analysis of the PFGE banding patterns was performed with the Fingerprinting II Informatix software package (Bio-Rad Laboratories, Hercules, CA). The similarity of the PFGE banding patterns was analyzed by the Dice coefficient, and cluster analysis was performed by the unweighted pair group method with arithmetic average (UPGMA). We applied a cutoff equivalent to 82% to group possibly genetically related isolates (34). Different PFGE types were designated by capital letters or numbers (35, 36, 38). Subtypes were designated by a number (indicating the number of bands that differed from the index strain) and primes when necessary (to distinguish among subtypes with the same number of bands but showing different banding patterns) (48).

Characterization of glycopeptide resistance. The Tn*1546* backbone was analyzed by PCR mapping, as previously described (38, 54). Conjugation experiments were performed by filter mating at a 1:1 donor-recipient ratio using *E. faecium* GE-1 and/or 64/3 and *E. faecalis* JH2-2 as recipient strains and vancomycin (6 mg/liter), rifampin (30 mg/liter), and fusidic acid (20 mg/liter) as antimicrobial selective markers. The genomic locations of *vanA* and *tcrB* were assessed by hybridization of I-CeuI and S1 nuclease (Takara Bio Inc., Shiga, Japan)-digested genomic DNA using intragenic *vanA*, *tcrB*, and 23S rRNA gene probes (16). Plasmid analysis included determination of size and content by PFGE of S1 nuclease-digested genomic DNA as previously described (16). Also, the identification of replication initiator proteins (rep) and maintenance systems (the toxin-antitoxin systems Axe-Txe and ω-ε-ζ and the partition module par_{pAD1}) was performed using recently developed PCR plasmid-typing methods, sequencing, and hybridization (25, 44). Restriction fragment length polymorphism (RFLP) analysis using EcoRI or ClaI enzyme was performed for plasmids of <150 kb (13). The plasmids were designated with Roman numerals (*E. faecalis*) or capital letters (*E. faecium*), following the nomenclature of previous studies in which some of the strains were initially reported (13). Hybridization experiments were performed by using the Gene Images AlkPhos Direct labeling and detection system (Amersham GB, GE Healthcare Life Sciences UK Limited). We referred to the rep sequences according to the plasmid type in which they were identified, as well by as the numeric nomenclature used by Jensen et al. (25).

RESULTS

Thirty-two (31 *E. faecium* [VREfm]; 1 *E. faecalis* [VREfs]) of the 41 VRE isolates studied were grouped in PFGE types highly similar to those of strains causing infection in hospitalized patients. They were identified as *E. faecium* CC5 (n = 30), *E. faecium* CC17 (n = 1), and *E. faecalis* CC2 (n = 1). Five other fecal isolates from healthy humans were classified as *E. faecium* ST18 (CC17), which is one of the predominant STs of the polyclonal subcluster CC17, although no similar PFGE types were observed among hospital VRE. The other four isolates were recovered from healthy humans, but their PFGE

or MLST profiles were not related to animal or hospital VRE. A detailed analysis of the isolates found in swine and healthy and hospitalized humans is provided in Table 2.

CC5 *E. faecium* carrying *vanA* on large plasmids (>150 kb) spread among human and swine hosts from the EU and the United States. Thirty *E. faecium* isolates clustering in CC5 (17 ST6, 6 ST5, 5 ST185, 1 ST147, and 1 ST493) were obtained from swine samples from Denmark, Portugal, Spain, Switzerland, and the United States, as well as from fecal samples from healthy Danes (1995 to 2008) (Table 2). Each ST comprised isolates recovered during a wide temporal and geographical frameshift that showed similar PFGE banding patterns (≤6 bands difference, >82% similarity [Fig. 1]): ST6 (1995 to 2008; 5 countries; subtypes A, A1, A1′, A1″, A1‴, and A2), ST5 (2003 to 2008; 2 countries; A1⁗, A3″, A3‴, and A5), ST147 (2003; 1 country; A5), ST185 (2001 to 2008; 3 countries; A3, A3′, A4, and A4′), and ST493 (2005; 1 country; A6). The isolates were classified as clonally related following interpretive criteria using PFGE (48) and taking into account the similarity among MLST profiles (all STs were single- or double-locus variants [SLVs or DLVs, respectively] of ST6, with the exception of ST493, which differs in three alleles). These strains were similar to a CC5 epidemic VRE clone widespread among swine from different EU countries since the mid-1990s, and they were considered to be the same clone (35). Comparison with large collections of hospital VRE revealed two clonally related isolates causing urinary tract infections in patients from different Portuguese hospitals (ST5, subtype A3⁗). These clinical isolates were not associated with a nosocomial outbreak.

All human and animal isolates expressed *tetM*-mediated tetracycline resistance, and most isolates (25/30) were also resistant to erythromycin (*ermB*), while none contained the putative virulence gene *esp* or *hyl.* They all carried a deleted variant of Tn*1546* previously designated type "D" and largely linked to swine hosts in different studies (26, 38, 54) and the gene *tcrB* coding for copper resistance, both located on large conjugative plasmids ranging from ca. 150 kb to 190 kb (see Fig. S1 in the supplemental material). All *vanA* plasmids carried a protein homologous to RepA of the recently sequenced megaplasmid pLG1 (GenBank accession number HM565183), which seems to be widespread among hospital *E. faecium* strains (29; A. R. Freitas and T. M. Coque, unpublished data).

CC17-ST132 *E. faecium* carrying Inc18-like *vanA* plasmids spread among human and swine hosts from Portugal. One ST132-VREfm isolate from swine ($n = 1$) recovered in Portugal in 2007 showed a PFGE type identical to that of two Portuguese isolates causing urinary tract infections recovered in 2002 (14) (Table 2 and Fig. 1). ST132 is an SLV of ST18, a predominant clone of the CC17 polyclonal subcluster. Although ST132 is not a predominant CC17 clone, isolates belonging to the ST have been recovered from unrelated patients hospitalized in Portugal and Tanzania, and it has been associated with a nosocomial outbreak described in Spain during an 18-month period, suggesting potential transmissibility (14, 39, 43, 53; http://efaecium.mlst.net).

All three isolates expressed resistance to ampicillin, erythromycin, and glycopeptides and high levels of resistance (HLR) to gentamicin and kanamycin due to the presence of *ermB*, *vanA*, *aac(6′)-aph(2″)*, and *aph(3′)-IIIa* genes. They also contained the *esp* gene, usually associated with a pathogenicity island of *E. faecium* (Table 2). Similar Tn*1546* backbones identified in these isolates (all carried IS*1216* within the *vanX-vanY* region and differed only upstream of *vanR*) were located on ca. 60-kb plasmids showing highly similar RFLP patterns (Fig. 2). Plasmid DNA hybridized with probes specific for homologues of replicases from the Inc18 plasmid pRE25 ($rep2_{pRE25}$; GenBank accession no. X92945) and pRUM ($rep17_{pRUM}$; GenBank accession no. AF507977). The *vanA* plasmid recovered from the clinical isolate contained a third rep highly homologous to pIP501 and other Inc18-like plasmids ($rep1_{pIP501}$; GenBank accession no. AJ505823) and was the only transferable vancomycin-resistant plasmid harbored by the clone.

Plasmids showing an RFLP pattern identical to that identified in ST132 strains have also been observed among strains isolated from hospital sewage and the Douro river in Portugal over a long time. Although they belong to ST368 and ST369, which are SLVs of ST132, they showed different PFGE types (data not shown).

Although no clonal relationships with clinical or animal isolates were observed for the two PFGE types corresponding to the five *E. faecium* ST18 (CC17) isolates recovered from a healthy human during a 5-year period, it is of interest to highlight the relationship of the genetic elements of these strains with others described above. Both clones expressed resistance to ampicillin, erythromycin, and tetracycline, while they differed in susceptibility to ciprofloxacin and resistance to high levels of gentamicin and streptomycin (Table 2). Two Tn*1546* variants, one containing an IS*Ef1* insertion, which is usually recovered from hospital VRE (previously designated PP5) (39), and the other associated with swine (type D), were linked to each PFGE type (Table 2), probably reflecting different acquisition events. The rep gene content was similar to that described above for other *E. faecium* CC17 isolates.

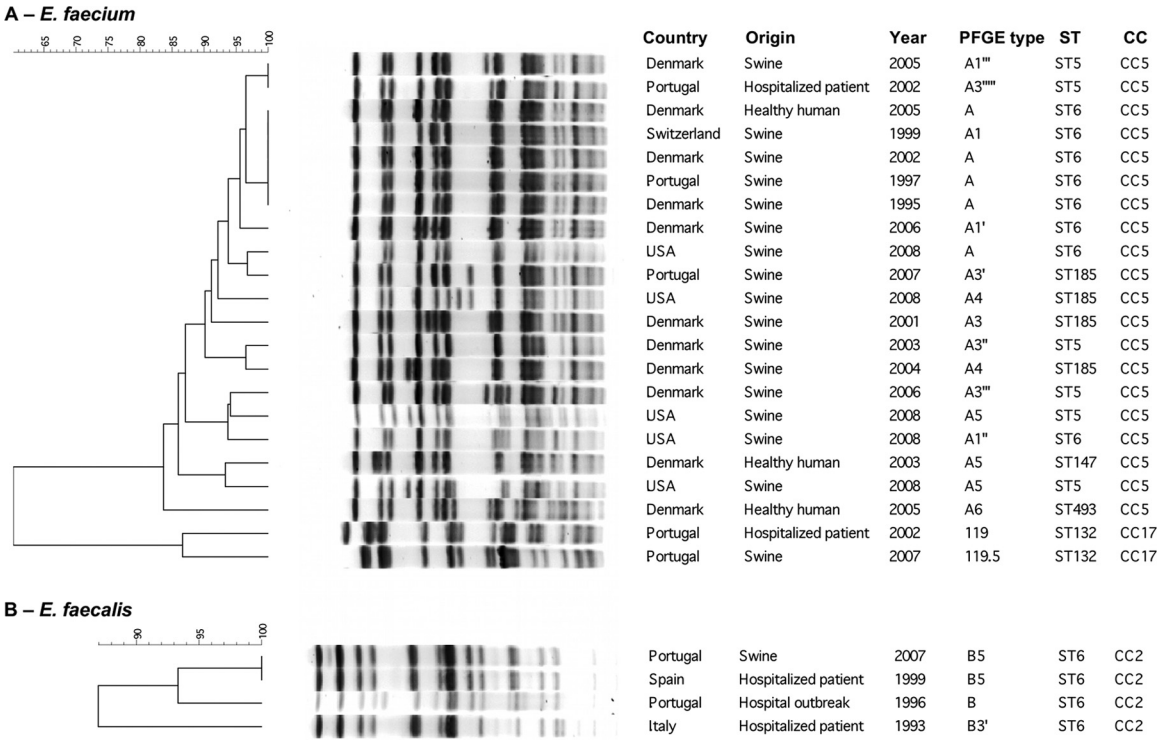
CC2-ST6 *E. faecalis* strains carrying pheromone-like *vanA* plasmids are disseminated among human and swine hosts in Europe. The VREfs isolate recovered from Portuguese swine showed a PFGE type identical to that of a multidrug-resistant ST6-CC2 *vanA E. faecalis* strain isolated from Portuguese, Italian, and Spanish hospitals since at least 1993 (15) (Fig. 1). They commonly expressed resistance to tetracycline, erythromycin, ciprofloxacin, and HLR to gentamicin, while HLR to kanamycin were seen and resistance to chloramphenicol was variable. The *vanA*, *tetM*, and *aac(6′)-aph(2″)* genes were detected in all four isolates, with the exception of the pig isolate, which lacked *ermB* but still contained *aph(3′)-IIIa* (Table 2). Like the majority of VREfs from Portuguese hospitals, the swine isolate did not contain *esp* (unpublished results).

A complete Tn*1546* backbone was identified in all CC2-ST6 isolates analyzed except the Portuguese clinical isolate, in which an IS*Ef1* insertion was identified (36). Plasmids carrying Tn*1546* ranged from 85 kb to 100 kb. The same ClaI-digested DNA pattern was observed among plasmids of ca. 100 kb from the Spanish clinical isolate (1999) and the Portuguese swine isolate (Fig. 2), which contained sequences homologous to those of replicases linked to pRE25 ($rep2_{pRE25}$) and to pheromone-responsive plasmids pBEE99 and pTEF2 ($rep9_{pCF10}$) (GenBank accession numbers NC_013533 and NC_004671, respectively).

TABLE 2. Epidemiological and genetic backgrounds of clonally related enterococcal isolates from humans and swine recovered in Denmark, Portugal, Spain, Switzerland, and the United States

Species and CC	ST	PFGE type	No. of isolates	Source	Country	Yr of isolation	Antibiotic resistance profile[g]	Antibiotic resistance genes	*tcrB*[h]	Putative virulence traits	Tn*1546* type[f]	Mating[h]	*vanA* plasmid	
													Size (kb)	RFLP[i]
E. faecalis CC2	ST6	B3′	1	Hospitalized patient (blood)	Italy	1993	VAN, TEC, TET, ERY, CIP, GEN, CHL	*vanA*, *tetM*, *ermB*, *aac(6′)-aph(2″)*	−	*agg*, *gel*	A	+	100	VI
		B[a]	1	Hospitalized patient (blood)	Portugal	1996	VAN, TEC, TET, ERY, CIP, GEN, KAN, CHL	*vanA*, *tetM*, *ermB*, *aac(6′)-aph(2″)*	−	*agg*, *gel*, *cyl*, *esp*	PP-4	+	85	I
		B5	1	Hospitalized patient (blood)	Spain	1999	VAN, TEC, TET, ERY, CIP, GEN, CHL	*vanA*, *tetM*, *ermB*, *aac(6′)-aph(2″)*	−	*agg*, *gel*	A	+	100	III
		B5	1	Piggery (manure)	Portugal	2007	VAN, TEC, TET, ERY, CIP, GEN, KAN, CHL	*vanA*, *tetM*, *aac(6′)-aph(2″)*, *aph(3′)-IIIa*	−	*agg*, *gel*	A	+	100	III
E. faecium CC17	ST132[b]	119[c]	2	Hospitalized patient (UTI[j])	Portugal	2002	VAN, TEC, AMP, ERY, CIP, GEN, KAN, Q-D	*vanA*, *ermB*, *aac(6′)-aph(2″)*, *aph(3′)-IIIa*	−	*esp*	PP-13	+	65	P
		119.5	1	Piggery (manure)	Portugal	2007	VAN, TEC, AMP, ERY, GEN, KAN	*vanA*, *aac(6′)-aph(2″)*, *aph(3′)-IIIa*	−	*esp*	PP-31	−	60	P1
E. faecium CC5	ST5	A3‴[d]	2	Hospitalized patient (UTI)	Portugal	2001–2002	VC, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	150	ND
		A1‴, A3″, A3‴	4	Slaughterhouse (feces)	Denmark	2003–2006	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D ($n = 2$)	+	150–170	ND
		A5	2	Piggery (animal feces)	United States	2008	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	170	ND
	ST6	A, A1′, A1″, A2	9	Slaughterhouse (feces)	Denmark	1995–2006	VAN, TEC, TET, (ERY)	*vanA*, *tetM*, *(ermB)*	+		D ($n = 3$)	+	145–180	ND
		A	1	Healthy humans	Denmark	2005	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	175	ND
		A[e]	1	Slaughterhouse (feces)	Portugal	1997	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	175	ND
		A1	1	Piggery (feces)	Switzerland	1999	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	150	ND
		A1′	2	Slaughterhouse (feces)	Spain	1998–2000	VAN, TEC, TET, ERY, KAN, STR	*vanA*, *tetM*, *ermB*, *aph(3′)-IIIa*	+		D	+	150	ND
		A, A1‴	3	Piggery (animal feces)	United States	2008	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D ($n = 2$)	+	170	ND
	ST147	A5	1	Healthy humans	Denmark	2003	VAN, TEC, TET	*vanA*, *tetM*	+		D	+	175	ND
	ST185	A3, A4′	2	Slaughterhouse (feces)	Denmark	2001–2004	VAN, TEC, TET, (ERY)	*vanA*, *tetM*, *(ermB)*	+		D	+	160–180	ND
		A3′	2	Piggery (soil)	Portugal	2007	VAN, TEC, TET	*van*, *tetM*	+		D	+	150	ND
		A4	1	Piggery (animal feces)	United States	2008	VAN, TEC, TET, ERY	*vanA*, *tetM*, *ermB*	+		D	+	170	ND
	ST493	A6	1	Healthy humans	Denmark	2005	VAN, TEC, TET, ERY, KAN	*vanA*, *tetM*, *ermB*, *aph(3′)-IIIa*	+		D	+	190	ND

[a] Representative isolate from a hospital outbreak strain widespread in six Portuguese hospitals in different cities (1996–2008).
[b] ST132 is a single-locus variant of ST18.
[c] Representative strain of two isolates causing single urinary tract infections in unrelated patients in one Porto hospital during 2002; only one was characterized.
[d] Clinical strain causing single urinary tract infections and disseminated in two hospitals during 2001–2002.
[e] Representative isolates of a strain widespread in Europe since the mid-1990s (35).
[f] The Tn*1546* designation is based on PCR mapping as described previously (38, 54). Briefly, type A corresponds to the whole Tn*1546* backbone; type D contains alterations within *orf1* and a point mutation at position 8234 in *vanX*; PP-4 contains an IS*Ef1* insertion within the *vanX-vanY* region; PP-13 and PP-31 have an IS*1216* insertion within the *vanX-vanY* region (PP-31 differs in the region upstream of *vanR*). The number of isolates included in the Tn*1546* typing is indicated when appropriate.
[g] VAN, vancomycin; TEC, teicoplanin; AMP, ampicillin; TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; GEN, high-level resistance to gentamicin; KAN, high-level resistance to kanamycin; STR, high-level resistance to streptomycin; CHL, chloramphenicol; Q-D, quinupristin-dalfupristin. Antibiotic designations in parentheses represent variable resistance to the indicated antibiotic.
[h] −, negative; +, positive.
[i] ND, not done.
[j] UTI, urinary tract infection.



Country	Origin	Year	PFGE type	ST	CC
Denmark	Swine	2005	A1'''	ST5	CC5
Portugal	Hospitalized patient	2002	A3''''	ST5	CC5
Denmark	Healthy human	2005	A	ST6	CC5
Switzerland	Swine	1999	A1	ST6	CC5
Denmark	Swine	2002	A	ST6	CC5
Portugal	Swine	1997	A	ST6	CC5
Denmark	Swine	1995	A	ST6	CC5
Denmark	Swine	2006	A1'	ST6	CC5
USA	Swine	2008	A	ST6	CC5
Portugal	Swine	2007	A3'	ST185	CC5
USA	Swine	2008	A4	ST185	CC5
Denmark	Swine	2001	A3	ST185	CC5
Denmark	Swine	2003	A3''	ST5	CC5
Denmark	Swine	2004	A4	ST185	CC5
Denmark	Swine	2006	A3'''	ST5	CC5
USA	Swine	2008	A5	ST5	CC5
USA	Swine	2008	A1''	ST6	CC5
Denmark	Healthy human	2003	A5	ST147	CC5
USA	Swine	2008	A5	ST5	CC5
Denmark	Healthy human	2005	A6	ST493	CC5
Portugal	Hospitalized patient	2002	119	ST132	CC17
Portugal	Swine	2007	119.5	ST132	CC17

Portugal	Swine	2007	B5	ST6	CC2
Spain	Hospitalized patient	1999	B5	ST6	CC2
Portugal	Hospital outbreak	1996	B	ST6	CC2
Italy	Hospitalized patient	1993	B3'	ST6	CC2

FIG. 1. Computer analysis of SmaI-digested genomic DNAs of representative vancomycin-resistant *E. faecium* (A) and *E. faecalis* (B) isolates from humans and swine. PFGE subtypes were established by visual analysis following the criteria of Tenover et al. (48). Computer analysis was performed with the Fingerprinting II Informatix software package (Bio-Rad Laboratories, Hercules, CA). The acquisition of the image was performed by using a Gel Doc XR camera (Bio-Rad Laboratories Inc.). The image was normalized by using four reference lanes of the low-range PFGE marker (0.13 kb to 1,018.5 kb; New England BioLabs, La Jolla, CA) as an external DNA size marker. The phylogenetic tree was subsequently constructed by use of the Dice coefficient and UPGMA clustering (optimization, 0.5%; band tolerance, 1.5%; threshold cutoff value set at 82%) (36). Year, year of isolation.

DISCUSSION

The present study suggests interhost transmission of particular VRE strains belonging to predominant enterococcal clonal complexes associated with hospital-acquired human infections (*E. faecium* CC17 and *E. faecalis* CC2) or swine colonization (*E. faecium* CC5) in several European countries. The recovery from swine in Europe and the United States of an *E. faecium* CC5 clone with the ability to either colonize humans or cause human infections is of concern, and it might be added to the list of clonal lineages of Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* ST398 or *Staphylococcus pseudintermedius* ST71, which are increasingly reported among animals and humans (46, 47). Although the origin and transmission routes of swine colonized by clonally related *E. faecium* CC5 isolates could not be established, inter- and intracountry food trade or dispersal of contaminated animals for food production cannot be ruled out. Contaminated imported chickens were suggested as the cause for clonal expansion of VREfs among poultry and pets in Japan and New Zealand during the last decade, although the isolates were not studied in detail at the genetic level (33, 40). Besides dispersal of animals in global markets, the selective pressure exerted by antibiotics (e.g., tetracyclines and β-lactams) or metals (e.g., copper) heavily used in veterinary medicine and husbandry may have contributed to the maintenance of VRE among swine farms and facilitated the horizontal transfer of conjugative plasmids to other enterococcal hosts (or other Gram-positive species serving as intermediates in the processes of horizontal gene transfer). Similarly to that described for *E. faecium* CC5, widespread clones of the predominant human enterococcal lineage *E. faecium* CC17 or *E. faecalis* CC2 have been recovered from companion and farm animals (4, 9, 13, 14).

Although some of the most common STs associated with *E. faecium* CC5 (ST5 and ST6), *E. faecium* CC17 (ST18), and *E. faecalis* CC2 (ST6) were detected, an unexpected diversity of STs and PFGE subtypes was observed within closely related *E. faecium* isolates belonging to CC5 and CC17. Examples of isolates showing the same or similar PFGE types but clustering in different STs have previously been described for both *E. faecalis* (13, 27) and *E. faecium* (7). It is known that large plasmids or integrative conjugative elements (ICEs), which are frequent in *E. faecium* (16, 21, 29, 55), can affect digested genomic DNA banding patterns (34, 49). In addition, a recent paper shows that mobilization of ICEs mediated by plasmids may also contribute to the diversity of housekeeping genes included in the MLST scheme (32). All these observations highlight not only the plasticity of enterococcal clones, which are able to evolve by diverse lateral transfer or mutational events, but also the potential difficulties in establishing epidemiological links among strains in some instances.

Confirming previous observations, specific Tn*1546* variants

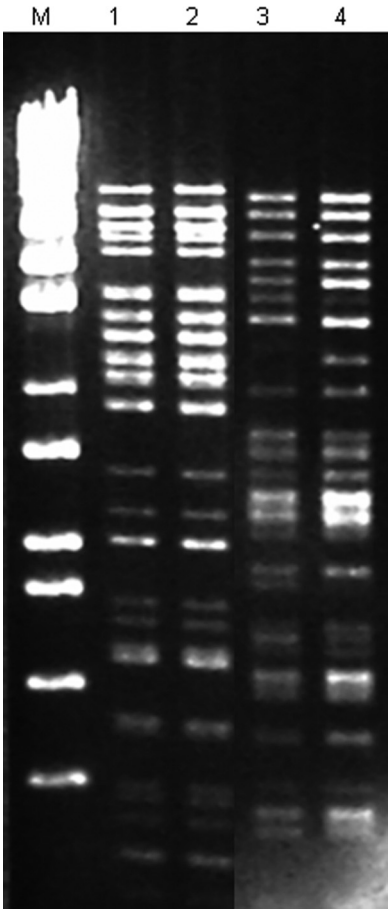


FIG. 2. ClaI-digested plasmid DNAs of VRE isolates recovered from hospitals and swine from different origins. Lane M, molecular marker ladder EcoT 14 I/BglII (Takara Bio Inc., Otsu, Japan); lane 1, *E. faecalis* ST6-CC2 (PFGE B5) from an outbreak strain recovered in Spain in 1999; lane 2, *E. faecalis* ST6-CC2 (PFGE B5) from a swine isolate recovered in Portugal in 2007; lane 3, *E. faecium* ST132-CC17 (PFGE 119.5) from a swine isolate recovered in Portugal in 2007; lane 4, *E. faecium* ST132-CC17 (PFGE 119) from a clinical isolate recovered in Portugal in 2002 that is representative of strains causing urinary tract infections in two unrelated patients during 2002.

were associated with VRE from humans or animals (10, 24, 38, 52, 54). The linking of such Tn*1546* variants with particular plasmid types from *E. faecium* (megaplasmids and mosaic plasmids containing replication proteins belonging to the RepA_N family, such as those of pLG1 or pRUM, and Inc18) or *E. faecalis* (pheromone-responsive plasmids) is also in agreement with some recent studies (12, 25, 29, 44, 56). Rosvoll et al. recently described the presence of *vanA*-Inc18 plasmids containing one or two rep (rep_1 and/or rep_2) among *E. faecium* strains from poultry and farmers in Norway and Italy (44), some of them closely related to the first *vanA*-Inc18 plasmid recovered in France in 1986 from a clinical isolate (45). Other studies have also described the presence of large plasmids carrying Tn*1546* among *E. faecium* isolates from hospitalized humans (2, 51) and swine (19). The recovery of resistant plasmids from clonally unrelated isolates from different sources over an extended period of time, such as that carried by the ST132 clone, is of concern, since it would reflect a wide spread and maintenance in both human and nonhuman hosts.

In summary, this study documents that enterococcal clones belonging to host-adapted clonal complexes of *E. faecium* (CC5 and CC17) and *E. faecalis* (CC2) are shared by swine and humans. The fact that these clones are able to colonize and cause human infections (and, in some instances, nosocomial outbreaks, such as *E. faecium* ST132 or *E. faecalis* ST6) confirms the relevance of reverse and alternative routes for dissemination of commensal and opportunistic bacteria. Multilayered molecular epidemiology studies will be required to understand the spread and evolution of clones and genetic elements encoding vancomycin resistance and overcoming the species barriers between humans and swine.

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